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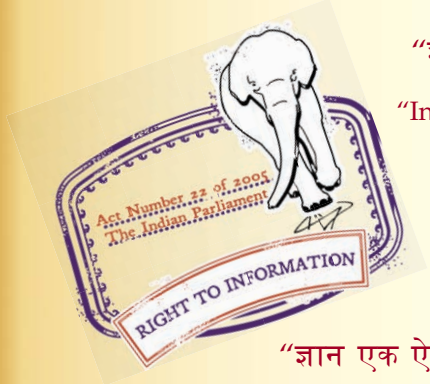
IS 11061 (1984): Code for preparation of VIBRIO CHOLERAE diagnostic sera [FAD 15: Food Hygiene, Safety Management and Other Systems]



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"Knowledge is such a treasure which cannot be stolen"

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IS : 11061 - 1984
(Reaffirmed 2005)

Indian Standard

CODE FOR PREPARATION OF *VIBRIO CHOLERAE* DIAGNOSTIC SERA

UDC 616·981·31-07 : 616·15-078-097·3 : 613·6

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Indian Standard

CODE FOR PREPARATION OF *VIBRIO CHOLERAE* DIAGNOSTIC SERA

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Indian Standard

CODE FOR PREPARATION OF *VIBRIO CHOLERAE* DIAGNOSTIC SERA

0. FOREWORD

0.1 This Indian Standard was adopted by the Indian Standards Institution on 20 September 1984, after the draft finalized by the Food Hygiene Sectional Committee had been approved by the Agricultural and Food Products Division Council.

0.2 The production of reliable diagnostic sera, by immunizing rabbits, demands close attention at all stages in the process. The sera produced should be of good quality and specific in nature. Since the production of the diagnostic sera is costly, the users must be made aware of the need to conserve this reagent.

0.3 In the preparation of this standard, considerable assistance has been derived from the Central Researeh Institute, Kasauli.

0.4 In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS : 2-1960*.

1. SCOPE

1.1 This standard specifies the method for raising, absorption and testing of various diagnostic sera used for serotyping of *Vibrio cholerae.*

2. SELECTION OF VACCINE STRAINS

2.1 In the production of diagnostic agglutinating sera the vaccines for immunization of rabbits must be prepared from a known classical strain.

*Rules for rounding off numerical values (*revised*).

For this purpose, the recommended strains (*see* Appendix A) can be obtained from the following centres:

a) National Salmonella & Escherichia Centre and National Collection of Type Cultures, Central Research Institute, Kasauli;

b) National Institute of Cholera and Enteric Diseases, Calcutta;

c) National Salmonella Centre, King George Medical College, Lucknow; and

d) National Salmonella Centre (Veterinary), Izatnagar.

2.2 It is advisible not to use wild and field strains for preparation of vaccines in the production of diagnostic sera.

3. PREPARATION OF VACCINES

3.1 Plate out the recommended strain (freeze dried) on a good quality nutrient agar (*see* IS : 6850-1973*) plate which has been well dried. Incubate overnight at 37°C. Observe the appearance of the colonies and if any rough colonies are visible, repeat the plating to obtain smooth colonies.

3.1.1 From a satisfactory plate select 5 well separate smooth colonies and test for the absence of auto-agglutination by slide agglutination in physiological saline (0·85 percent) and 1 : 500 acriflavine aqueous solution.

3.1.2 Subculture all 5 colonies in 10 ml of nutrient broth and incubate at 37°C.

3.1.3 After 4 to 6 hours, inoculate a nutrient agar plate (diameter 145 mm) or a rolled whisky agar bottle with 1 ml or 5 ml of the nutrient broth cultures respectively; spread over the surface of the plate/bottle and incubate overnight at 37°C.

3.1.4 Harvest the growth in 2 ml of physiological saline (0·85 percent) so as to obtain a thick suspension.

3.1.5 Steam at 100°C for 2 hours 30 minutes and then cool.

3.1.6 Drop by drop add the thick steamed suspension to 20 ml of buffered formol saline (*see* Appendix B) until the capacity is about $3\,000 \times 10^6$ organisms per ml. This is the vaccine suspension and usually remains satisfactory for six months, if stored at 4°C.

3.2 Auto-agglutination Test

3.2.1 Before use the vaccine suspension shall be tested for auto-agglutination.

*Specification for agar, microbiological grade.

3.2.2 Dilute the suspension to 400 × 10^6 organisms per ml using buffered formol saline and place 0·5 ml into each of 4 Dreyer's tubes. Incubate at 50 to 52°C for 18 hours and check for auto-agglutination.

3.2.3 The vaccine suspension should be tested in tubes against a homologous serum and should give at least standard agglutination at the stated titre of the serum.

3.2.4 Standard *Vibrio cholerae* serum used for the tube agglutination tests are prepared by immunizing rabbits. These should have a sufficient titre (1/600) and should show no cross reactions with heterologous antigens.

4. IMMUNIZATION AND BLEEDING SCHEDULES

4.1 Selection of Rabbits — Disease - free rabbits weighing 1·5 to 2 kg are test bled for pre-immunity agglutinating titres. If any rabbit serum shows agglutination against *Vibrio cholerae* that animal is not suitable for raising the serum and is discarded.

4.2 Immunization — Rabbits are injected with the immunizing suspension (**3.1.6**) according to the schedule shown below :

Day	*Suspension in ml*
First	0·25
Third	0·5
Sixth	1·0
Ninth	1·5
Twelvth	2·0
Sixteenth	Bleeding

4.3 Bleeding Schedule — The rabbits are test bled on the 4th day of the last injection. If the titre is found suitable, the rabbits are bled out by cardiac puncture as follows:

a) 20 ml of blood is withdrawn from the heart 2-5 days after the test bleeding;

b) Another 20 ml bleeding 2-5 days later; and

c) The rabbits are finally bled by exsanguination 2-5 days after the second bleeding.

4.3.1 Blood is collected into 150 × 16 mm test-tubes or other suitable glass containers), which have been washed and rinsed thoroughly. Allow the blood to clot; first placing in a 37°C incubator for 2 hours and then in a refrigerator (4°C) overnight.

4.3.2 Remove the serum on the following day. Any serum which shows haemolysis should be discarded. The serum from the three bleeds is pooled.

4.3.3 After being tested and any necessary absorptions having been completed the serum is seitz filtered or membrane filtered and preserved by adding 0·4 percent phenol saline.

4.3.4 The serum shall be stored in the refrigerator at 4°C.

NOTE – Most antigens produce an adequate response after one course of injections. Repeated immunization courses often produce sera with cross reactions to heterologous antigens. Such sera require extensive absorptions to ensure specificity and therefore repeated immunization courses are not recommended.

5. TESTING OF SERUM

5.1 The serum is tested for homologous and heterologous antibody titre by tube agglutination. The results of this testing will determine the extent of absorption required.

6. ABSORPTION

6.1 The sera showing heterologous agglutination require to be absorbed. The absorbing strain(s) should be smooth after giving 3 passages in motility test medium. The organisms for absorption are grown in nutrient agar plates (145 mm) or whisky agar bottles and the growth harvested through normal saline and heated at 100°C for 2 hours 30 minutes, centrifuged and the supernatant discarded. The serum under absorption is added to the sediment and mixed thoroughly. It is then kept in a water bath at 52°C for 2 hours and centrifuged. The clear supernatant serum is pipetted off and tested for agglutination. If the serum gives agglutination against a heterologous culture, second absorption is done as before. After the absorption procedure, the serum is seitz filtered or membrane filtered and agglutinating titre is found out.

NOTE – Serum to be discarded if heterologous titre persists after second absorption.

7. FINAL TESTING OF ABSORBED SERA

Absorbed serum should give the under mentioned titre.

7.1 In Case of *Vibrio Cholerae* Inaba Serum

Vibrio cholerae Inaba = 160 or above (Homologous)
Vibrio cholerae Ogawa = 20 Neg (Heterologous)
Vibrio cholerae Rough = 20 Neg (Heterologous)

7.2 In Case of *Vibrio Cholerae* Ogawa Serum

Vibrio cholerae Ogawa = 160 or above (Homologous)
Vibrio cholerae Inaba = 20 Neg (Heterologous)
Vibrio cholerae Rough = 20 Neg (Heterologous)

7.3 In Case of *Vibrio Cholerae* Rough Serum

Vibrio cholerae Rough = 160 or above (Homologous)
Vibrio cholerae Inaba = 20 Neg (Heterologous)
Vibrio cholerae Ogawa = 20 Neg (Heterelogous)

7.4 In Case of *Vibrio Cholerae* Non-differential Serum

Vibrio cholerae Inaba = 160 or above
Vibrio cholerae Ogawa = 160 or above
Vibrio cholerae Rough = 20 negative

8. PRESERVAION AND STORAGE OF SERA

8.1 After testing and any necessary absorptions the serum is seitz filtered or membrane filtered. This is then preserved in 0·4 percent phenol.

8.2 The sera are stored in the dark at 4°C and usually the titre remains steady. They must not be frozen. However, sometimes the homologous titre falls and it is impossible to predict which serum will behave in this manner. The fall in titre may be abrupt but in general, absorbed sera show this tendency more frequently than unabsorbed sera.

8.3 Before use or issue the titre of the Stock serum should be determined and the serum diluted with 0·4 percent phenol to give a titre of about 1 in 160.

8.4 The titre is then re-assessed by tube agglutination. In addition to giving a satisfactory titre the diluted serum must give a rapid agglutination when tested by slide agglutination with an appropriate suspension of the homologous strain.

8.5 If several bottles of diluted serum are being produced, the dilution is made in bulk and the diluted serum is distributed in 1 to 2 ml amounts into suitable sterile ampoules or vials. Each ampoule/vial is labelled showing the type of serum, titre and date of filling.

8.6 One bottle from each batch is retained as a reference. It is difficult to give a reliable figure for the active life of diluted sera but unopened bottles if stored in dark at 4°C should remain suitable for one year. These sera must *not* be frozen because they contain phenol.

9. LABELLING OF *VIBRIO CHOLERAE* SERA

9.1 Absorbed *Vibrio Cholerae* **Ogawa Sera** — It should be labelled as *Vibrio cholerae* Ogawa sera. It will agglutinate strains of *Vibrio cholerae* Ogawa and will not agglutinate strains of *Vibrio cholerae* Inaba when tested by the slide agglutination test.

9.2 Absorbed *Vibrio Cholerae* **Inaba Sera** — It shall be labelled as *Vibrio cholerae* Inaba sera. It will agglutinate strains of *Vibrio cholerae* Inaba and will not agglutinate strains of *Vibrio cholerae* Ogawa when tested by the slide agglutination test.

9.3 Absorbed *Vibrio Cholerae* **Non-differential Serum** — It shall be labelled as *Vibrio cholerae* non-differential sera. It shall agglutinate with strains of *Vibrio cholerae* Inaba and *Vibrio cholerae* Ogawa when tested by slide agglutination test.

9.4 Absorbed *Vibrio Cholerae* **Rough Serum** — It shall be labelled as *Vibrio cholerae* Rough Serum. It shall agglutinate with *Vibrio cholerae* rough strains but not with *Vibrio cholerae* Inaba and *Vibrio cholerae* Ogawa strains.

9.5 Final Label — The bottles (or ampoule) containing *Vibrio cholerae* agglutinating serum shall be labelled to give the following information :

a) Name of the manufacturer and manufacturing licence number, if any;

b) Name of the serum shall be written as indicated above;

c) Quantity, titre of the serum, the preservative used, if any, and temperature of storage;

d) Batch (or lot) number, manufacturing and expiry date;

e) Label should have the words 'FOR LABORATORY USE ONLY'; and

f) Not to be frozen, to be stored at 4 to 8°C.

10. TEST FOR STERILITY

10.1 The sera should be suitably tested for sterility.

11. RECORDS TO BE MAINTAINED BY THE MANUFACTURER

11.1 It shall be mandatory for the manufacturer to maintain viable strains of *Vibrio cholerae* used for preparing *Vibrio cholerae* diagnostic agglutinating sera. Such viable strains shall be made available. on demand, to evaluation/control laboratories. The manufacturer shall maintain detailed records, permanently, of the procedures used for maintaining the cultures of bacteria, for preparation of each batch (lot) of serum and results of titrations as obtained in production and before bottling (ampuling) for supply. Such records shall be made available, on demand, to evaluation/control laboratories.

It is desirable that the manufacturer should submit sera from each batch (lot) for evaluation and satisfactory report to evaluation/control laboratories before marketing/supplying the sera.

APPENDIX A

(Clause 2.1)

VIBRIO CHOLERAE STRAINS FOR IMMUNISATION AND ABSORPTION

NAME OF SERUM	RAISING STRAIN	ABSORBING STRAINS
Vibrio cholerae Inaba serum	*Vibrio cholerae* Inaba No 569/B	1. *Vibrio cholerae* Ogawa S/121/58
		2. *Vibrio cholerae* Ogawa B-53-2
		3 *Vibrio cholerae* Ogawa 41
Vibrio cholerae Ogawa serum	*Vibrio cholerae* Ogawa B-53-2	1. *Vibrio cholerae* Inaba 49520
		2. *Vibrio cholerae* Inaba 569/B
		3. *Vibrio cholerae* Inaba B-53-1
Vibrio cholerae Rough serum	*Vibrio cholerae* Rough B-53-3	1. *Vibrio cholerae* Inaba 49520
		2. *Vibrio cholerae* Ogawa S/121/58

APPENDIX B

(Clause 3.1.6*)*

PREPARATION OF BUFFERED FORMOL-SALINE (BFS)

a) Stock BFS 2·5 percent

Commercial formalin (40 percent)	50 ml
Physiological saline	2 000 ml

Adjust *p*H to 7·6 by addition of Na_2HPO_4 crystals

b) Working BFS 0·25 percent

Stock (2·5 percent BFS)	40 ml
Physiological saline	360 ml